Thermal destruction of *Listeria monocytogenes* during liver sausage processing

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We studied the inactivation of Listeria monocytogenes *during the thermal processing of liver sausage. Liver sausage emulsion was prepared and inoculated with* L. monocytogenes *Scott A to yield an initial population of* c. 10^9 g^{-1}. *The inoculated emulsion was stuffed into large diameter, moisture-proof fibrous casings and cooked in a smokehouse. Individual sausages were removed as the product reached various predetermined internal temperatures, and duplicate core samples were analyzed for viable* L. monocytogenes *by surface plating onto tryptose agar. The viable count remained unchanged in product heated to 140°F. In product heated to 145°F, the number of viable* L. monocytogenes *decreased and at 155°F, no viable* L. monocytogenes *were detected. Thus, liver sausage and other large diameter non-fermented sausage product heated to 155°F should be free of viable* L. monocytogenes.

Introduction

Listeria monocytogenes is a psychrotrophic human pathogen which is widely found in the environment. The organism can be readily isolated from water, vegetation, sewage and a variety of warm-blooded animals (Gray and Killinger 1966). It can also be isolated from various raw foods such as milk, poultry, red meat and seafoods (Johnson et al. 1990, Pearson and Marth 1990).

L. monocytogenes can be destroyed by many of the heat treatments used to process food products such as HTST milk pasteurization (Farber 1989, Lovett et al. 1990), ice cream mix pasteurization (Holsinger et al. 1992) and processing frankfurter emulsion to 160°F (Zaika et al. 1990). Previous studies from this laboratory (Bhaduri et al. 1991) investigated the thermal resistance of *L. monocytogenes* during the processing of liver sausage slurry and this permitted the determination of *D*-values for the organism in this system. In the present study, we report on the fate of *L. monocytogenes* during the thermal processing of liver sausage prepared under commercial process conditions in a smokehouse to define the final internal temperature which must be reached to ensure a product free of the organism.

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Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

Materials and Methods

Culture

Listeria monocytogenes Scott A (originally obtained from J. Hunt, FDA, Cincinnati, OH) was used throughout these studies. The stock culture of the organism was carried in Brain heart infusion broth (BHI; Difco, Detroit, MI) tubes held at 5°C. The culture was transferred at monthly intervals to ensure viability. For thermal destruction studies in sausages, the organism was grown first in a 1 litre Erlenmeyer starter flask containing 200 ml BHI broth with 0·3% added glucose and incubated for 20 h at 37°C with shaking (150 rpm). Ten flasks of BHI-glucose (200 ml in 1 litre Erlenmeyer flasks) were each inoculated with 20 ml from the starter flask and incubated as above. The cells were harvested by centrifugation (16 300 $\times g$, 5°), washed twice with sterile 0·1 M potassium phosphate buffer (pH 7·2) as done previously for inactivation studies on *Staphylococcus aureus* (Smith et al. 1982), and resuspended in *c.* 20–25 ml sterile distilled water.

Preparation of liver sausage

Standard liver sausage was prepared as described by Komarik et al. (1974) and Kramlich et al. (1973). Product was prepared in 10 kg batches as follows: fresh pork liver, 5 kg; 50/50 pork trimmings (coarse ground, ½" plate), 5 kg; NaCl, 250 g (2·5%); sucrose 100 g; $NaNO_2$, 1·56 g; and spice mixture, 52·34 g. The spice mixture contained: black pepper, 25 g; nutmeg, 6·25 g; clove, 6·25 g; ginger, 6·25 g; allspice, 6·25 g; onion powder, 1·56 g; and garlic powder, 0·78 g. The spice mixture was sterilized in a Cesium-137 source (30 kGy at 25°C) prior to use. Liver sausage emulsion was prepared as follows: the liver-pork trimmings were chopped in a Schnell Universal Kutter (Hobart, Model HC14–450, Troy, OH) until the mixture bubbled (about 10 min), then the rest of the ingredients were added and the mixture chopped another 5 min. The emulsion was then taken to the laboratory to be inoculated with the culture of *L. monocytogenes*. Duplicate experiments were performed on separate days.

Inoculation of liver sausage emulsion

The prepared emulsion was inoculated with the resuspended *Listeria* culture (with *c.* 5 ml green food dye added; previous work (Zaika et al. 1990) indicated that the green food dye had no adverse effect on the organism. Use of the green food dye ensured complete mixing of the culture and emulsion and aided in the clean-up and sanitation of equipment) and the culture mixed in by hand in a large sterile dishpan. All contaminated equipment, gloves, laboratory coats, etc., were disinfected either by autoclaving or treatment with a bacteriocidal sanitizer. After sampling, unneeded *Listeria*-containing sausage material was autoclaved before disposal. Routine pathogen control procedures were employed to ensure safety of laboratory personnel and prevent contamination of the laboratory environment. After the *Listeria* culture was mixed in, the inoculated liver sausage emulsion was stuffed into moisture proof fibrous casings (5 N; 13·33 cm flat diameter; Teepak, Danville, IL) to yield sausages approximately 1 kg in weight (*c.* 33 cm long). The ends of the casings were tied by hand.

Heating the inoculated liver sausages

The *L. monocytogenes*-containing sausages were heated, without smoke, in a temperature and humidity controlled smokehouse (Koch, Model MP-2; Koch Supplies, Inc., Kansas City, MO) set as the following sequential conditions: 160 min at 165°F dry bulb (DB) and 140°F wet bulb (WB); 50 min at 170°F DB and 140°F WB; and 185°F DB and 175°F WB until the final processing temperature was attained. Internal product temperature was monitored continuously with a thermocouple inserted into the geometric center of a sausage. As the internal temperature reached certain predetermined values, a sausage was removed from the smokehouse. One 50 g 1" center core [taken after the rounded end portion (*c.* 2") was cut off and discarded] was aseptically removed from each end of the sausage (designated A and B in Fig. 1), each placed in separate Stomacher filter bags, 200 ml cooled (in an ice bath) 0·1% peptone water added, and sausage-peptone water emulsion was blended for 2 min in a Stomacher 400 laboratory mixer. The sausage was sampled immediately upon removal from the smokehouse and thus rapid cooling took place during preparation of the initial 1:5 slurry. This procedure for sample cooling was initiated to eliminate previously observed non-

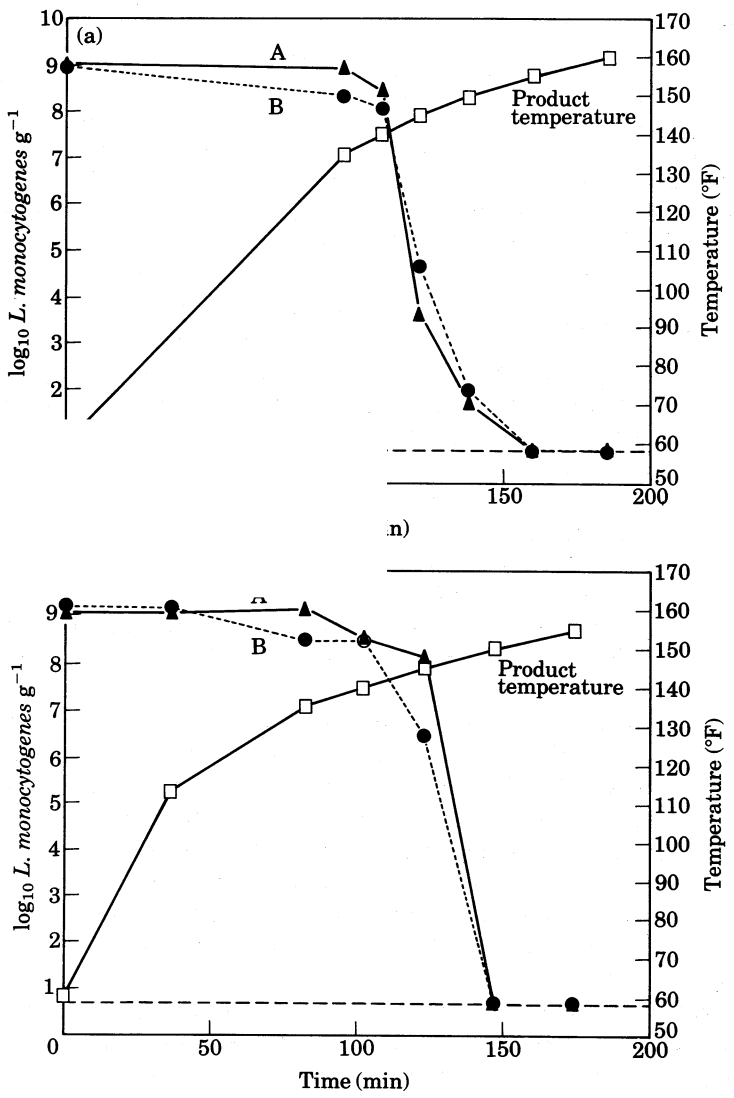

Fig. 1. Effect of heat processing on the viable population levels of *L. monocytogenes* inoculated into liver sausage. In the individual experiments, A and B represent samples taken from opposite ends of the same sausage. Experiments I and II represent individual experiments done on different days. (Dashed line represents the low limit of detection.) (a) Experiment I. (b) Experiment II.

reproducible thermal destruction plots caused by different amounts of continued killing that occurred if the whole sausages were cooled before sampling. Thus, the data presented represent a conservative estimate of the thermal destruction of *L. monocytogenes* which would occur under commercial conditions since further lethality would be expected during the normal cool-down time.

Determination of L. monocytogenes *in liver sausage*

The number of *L. monocytogenes* which survived heating to different internal temperatures was determined by surface plating the initial 1 : 5 slurry or further dilution(s) made in 0·1% peptone water onto tryptose agar (Difco). Plates were counted after 48 h at

37°C. This non-restrictive medium was used to ensure recovery of all (both injured and non-injured) *L. monocytogenes*; the identity of surviving colonies was verified as described previously by Zaika et al. (1990). The count of the raw emulsion before the addition of the *L. monocytogenes* culture was *c.* 10^4–10^5/g and consisted of Gram-negative rods. These were not encountered on any of the recovery plates.

Results and Discussion

Most thermal destruction studies of foodborne pathogens are done in model systems. In this study, we inoculated liver sausage emulsion with *L. monocytogenes* and then processed the sausages under conditions approximating commercial operations. Data from two separate experiments are presented in Fig. 1 (a,b). Both experiments show similar results: essentially no decrease in the number of viable *L. monocytogenes* was observed until the product temperature reached 145°F. At 150°F, there was a very marked decrease in viable *L. monocytogenes* and at 155°F, the number of viable *L. monocytogenes* was decreased to below the lower limit of detection. The data from an individual experiment [Experiment I, Fig. 1(a)] were analyzed using the degree minute technique (an integration procedure which combines time and temperature, see Zaika et al. 1990). Plotting of $\log_{10}$ *L. monocytogenes*/g vs total heat treatment in degree-min (not shown) gave a response similar to the simple plot of $\log_{10}$ *L. monocytogenes*/g vs time shown in Fig. 1.

Examination of thermal resistance data for *L. monocytogenes* Scott A from the literature indicated a range of $D_{60°C}$-values from 1·62 min for ground beef (Schoeni et al. 1991) to 8·32 min for ground beef slurry (Gaze et al. 1989); *Z*-values show a narrow range: 5·98°C for ground beef slurry (Gaze et al. 1989) to 8·4°C for blue crabmeat (Harrison and Huang 1990). Bhaduri et al. (1991) reported a *D*-value of 2·42 min at 60°C for Scott A in liver sausage slurry and a *Z*-value of 6·2°C. The thermal resistance of *L. monocytogenes* Scott A appears similar in several different food systems and thus the thermal destruction of high numbers of the organisms should occur during the heating step of liver sausage processing. Since liver sausage is generally processed to temperatures between 152–155°F, attainment of this final heating temperature should destroy any *L. monocytogenes* present on the starting meats. A brief holding at this temperature would further ensure a product free of viable *L. monocytogenes*. The data presented here should be applicable to other large diameter non-fermented sausage products. Preliminary studies (data not shown) indicated the response of *L. monocytogenes* inoculated into an all-beef sausage product was similar to that in liver sausage: constant numbers up to 140°F followed by a rapid decrease in viable count as the product temperature reached 145° and 150°F, with no viable *L. monocytogenes* in product heated to 155°F.

References

Bhaduri, S., Smith, P. W., Palumbo, S. A., Turner-Jones, C. O., Smith, J. L., Marmer, B. S., Buchanan, R. L., Zaika, L. L. and Williams, A. C. (1991) Thermal destruction of *Listeria monocytogenes* in liver sausage slurry. *Food Microbiol.* **8**, 75–78.

Farber, J. M. (1989) Thermal resistance of *Listeria monocytogenes* in foods. *Intl. J. Food Microbiol.* **8**, 285–291.

Gaze, J. E., Brown, G. D., Gaskell and Bank, J. G. (1989) Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef steak and carrot. *Food Microbiol.* **6**, 251–259.

Gray, M. L. and Killinger, A. H. (1966) *Listeria monocytogenes* and listeric infections *Bacteriol. Rev.* **30**, 309–382.

Harrison, M. A. and Huang, Y.-W. (1990) Thermal death times for *Listeria monocytogenes* (Scott A) in crabmeat. *J. Food Protect.* **53**, 878–880.

Holsinger, V. H., Smith, P. W., Smith, J. L. and Palumbo, S. A. (1992) Thermal destruction of *Listeria monocytogenes* in ice cream mix. *J. Food Protect.* **55**, 234–234.

Johnson, J. L., Doyle, M. P. and Cassens, R. G. (1990) *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products: a review. *J. Food Protect.* **53**, 81–91.

Komarik, S. L., Tressler, D. K. and Long, L. (1974) *Food products formulary.* Vol. I. *Meats, poultry, fish, shellfish*. Westport, CT, The Avi Publishing Company.

Kramlich, W. E., Pearson, A. M. and Tauber, F. W. (1973) *Processed Meats*. Westport, CT, The Avi Publishing Company.

Lovett, J., Wesley, I. V., Vandermaaten, M. J., Bradshaw, J. G., Francis, D. W., Crawford, R. G., Donnelly, C. W. and Messer, J. W. (1990) High temperature short-time pasteurization inactivates *Listeria monocytogenes. J. Food Protect.* **53**, 734–738.

Mackey, B. M., Pritchet, C., Norris, A. and Mead, G. C. (1990) Heat resistance of *Listeria*: strain differences and effect of meat type and curing salts. *Lett. Appl. Microbiol.* **10**, 251–255.

Peason, L. J. and Marth, E. H. (1990) *Listeria monocytogenes*—threat to a safe food supply: a review. *J. Dairy Sci.* **73**, 912–928.

Schoeni, J. L., Brunner, K. and Doyle, M. P. (1991) Rates of thermal inactivation of *Listeria monocytogenes* in beef and fermented sausages. *J. Food Protect.* **54**, 334–337.

Smith, J. L., Benedict, R. C. and Palumbo, S. A. (1982) Protection against heat injury in *Staphylococcus aureus* by solutes. *J. Food Protect.* **45**, 54–58.

Zaika, L. L., Palumbo, S. A., Smith, J. L., Del Corral, F., Bhaduri, S., Jones, C. O. and Kim, A. H. (1990) Destruction of *Listeria monocytogenes* during frankfurter processing. *J. Food Protect.* **53**, 18–21.